

Claims:

1. Hepatitis C virus (HCV) vaccine comprising at least two epitopes, each from a different hotspot epitope, wherein a hotspot epitope is defined as an epitope containing peptide selected from the group consisting of

KFPGGGQIVGGVYLLPRRGPRLGVRATRK,
GYKVLVLNPSVAAT,
AYAAQGYKVLVLNPSVAAT,
DLMGYIP (A/L) VGAPL,
GEVQVVSTATQSFLATCINGVCWTV,
HMWNFISGIQYLAGLSTLPGNPA,
VDYPYRLWHYPCT (V/I) N (F/Y) TIFK (V/I) RMYVGGVEHRL,
AAWYELTPAETTVRLR,
GQGWRL LAPITAYSQQTRGLLGCIIV,
IGLGKVLVDILAGYGAGVAGALVAFK,
FTDNSSPPAVPQTFQV,
LED RDRSELSPLLLSTTEW,
YLVAYQATVCARAQAPPPSWD,
MSTNPKPQRKTKRNTNR,
LINTNGSWHINRTALNCNDSL,
TTILGIGTVLDQAET,
FDS (S/V) VLCECYDAG (A/C) AWYE,
ARLIVFPDLGVRVCEKMALY,
AFCSAMYVGDL CGSV,
GVLFGLAYFSMVG NW,
VVCCSMSYTWTGALITPC,
TRVPYFVRAQGLIRA and
TTLLFNILGGWVAAQ.

2. HCV vaccine according to claim 1 comprising at least three, especially at least four epitopes, each from a different hotspot epitope.

3. HCV vaccine according to claim 1 comprising at least five epitopes, each from a different hotspot epitope.

4. HCV vaccine according to claim 1 comprising at least six epitopes, each from a different hotspot epitope.

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5. HCV vaccine according to any one of claims 1 to 4, characterised in that the epitopes are selected from the following groups:

KFPGGGQIVGGVYLLPRRGPRLGVRATRK, KFPGGGQIVGGVYLLPRRGPRL,
 YLLPRRGPRL, LPRRGPRL, GPRLGVRAT, RLGVRATRK;
 GYKVLVLNPSVAAT, AYAAQGYKVL, AYAAQGYKVLVLNPSVAAT;
 DLMGYIPAV, GYIPLVGAPL, DLMGYIPLVGAPL;
 CINGVCWTV, GEVQVVSTATQSFLAT, GEVQVVSTATQSFLATCINGVCWTV;
 HMWNFISGIQYLAGLSTLPGNPA, MWNFISGIQYLAGLSTLPGN, NFIS-
 GIQYLAGLSTLPGNPA, QYLAGLSTL, HMWNFISGI;
 VDYPYRLWHYPCTVNFTIFKVRMYVGGVEHRL, DYPYRLWHYPCTVNFTIFKI,
 DYPYRLWHYPCTVNFTIFKV, VDYPYRLWHYPCTVNFTIFKIRMYVGGVEHRL,
 DYPYRLWHYPCTVNFTIFKI, DYPYRLWHY, TVNYTIFKI, TINYTIFK,
 TVNFTIFKV, HYPCTVNFTI, HYPCTVNFTI, RMYVGGVEHR;
 AAWYELTPAETTVRLR, TPAETTVRL;
 GWRL LAPITAYSQQTRGLLGCIV, TAYSQQTRGLLGCIV, TAYSQQTRGLLG, GQGWRL-
 LAPITAYSQ, RLLAPITAY, GQGWRL LAPITAYSQQTRGLLGCIV, GQGWRL LAP-
 ITAYSQQTRGLLG, AYSQQTRGLL, AYSQQTRGL; IGLGKVLVDILAGYGAGVAGAL-
 VAFK, ILAGYGAGV, VAGALVAFK, GYGAGVAGAL;
 VVCCSMSYTWGTALITPC, SMSYTWGTALITP, SMSYTWGTAL, SYTWGTALI;
 FTDNSSPPAVPQTFQV;
 LEDRDRSELSPLLLSTTEW, LEDRDRSELSPLLLST, RSELSPLLL, ELSPLLLST,
 DRDRSELSPL, LEDRDRSEL, LEDRDRSEL;
 YLVAYQATVCARAQAPPPSWD, YLVAYQATV;
 MSTNPKPQRKTKRNTNR, PQRKTKRNTNR, QRKTKRNTN, RKTKRNTNR, MSTNPKPQR,
 MSTNPKPQK;
 LINTNGSWHINRTALNCNDSL, NGSWHINRTALNCNDSL, LINTNGSWHI, RTALNCND-
 SL, LINTNGSWHINRTALN, SWHINRTALN;
 TTILGIGTVLDQAET, TTILGIGTV, TILGIGTVL;
 FDSSVLCECYDAGAAWYE, FDSSVLCECYDAGCA, VLCECYDAGA, VVLCECY-
 DAGAAWYE;
 ARLIVFPDLGVRVCEKMALY, ARLIVFPDL, RLIVFPDLGV, RVCEKMALY,
 AFCSAMYVGDL CGSV;
 GVLFGLAYFSMVGW;
 TRVPYFVRAQGLIRA;
 TTLFNILGGWVAAQ, LLFNILGGWV.

6. HCV vaccine according to any one of claims 1 to 5 characterised in that it comprises at least one epitope from at least three, preferably at least four of the following hotspot epi-

topes:

KFPGGGQIVGGVYLLPRRGPRLGVRATRK,
AYAAQGYKVLVLNPSVAAT,
DLMGYIP (A/L) VGAPL,
GEVQVVSTATQSFLATCINGVCWTV and
HMWNFISGIQYLAGLSTLPGNPA.

7. HCV vaccine according to any one of claims 1 to 6 characterised in that it comprises at least one epitope from at least two, preferably at least three, especially at least four of the following hotspot epitopes:

VDYPYRLWHYPCT (V/I) N (F/Y) TIFK (V/I) RMYVGGVEHRL,
AAWYELTPAETTVRLR,
GQGWRL LAPITAYSQQTRGLLGCIIV,
IGLGKVLVDILAGYGAGVAGALVAFK,
FTDNSSPPAVPQTFQV,
LEDRDRSELSPLLLSTTEW,
YLVAYQATVCARAQAPPSWD,
MSTNPKPQRKTKRNTNR and
LINTNGSWHINRTALNCNDSL.

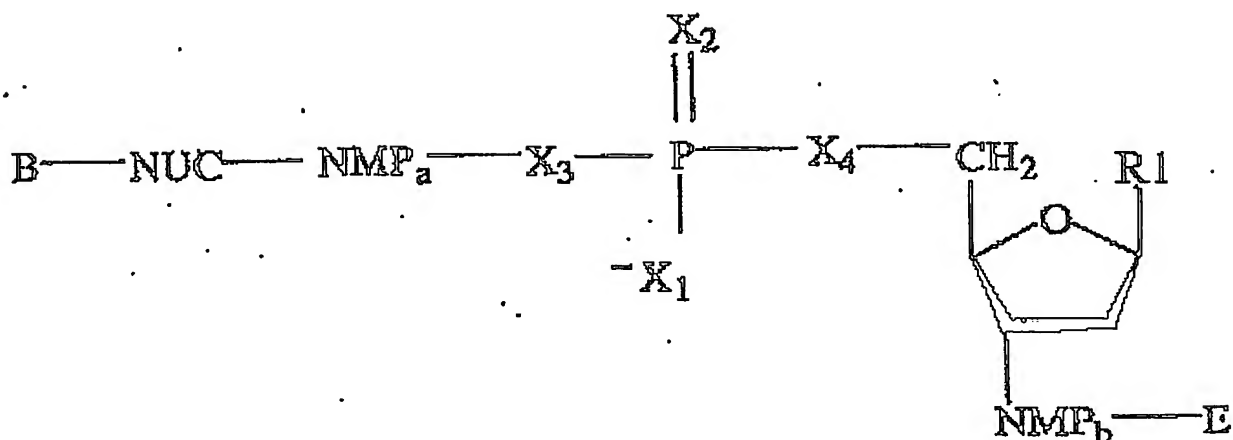
8. HCV vaccine according to any one of claims 1 to 7 characterised in that it comprises at least one epitope from at least two, preferably at least three, especially at least four of the following hotspot epitopes:

TTILGIGTVLDQAET,
FDS (S/V) VLCECYDAG (A/C) AWYE,
ARLIVFPDLGVRVCEKMALY,
AFCSAMYVGDL CGSV,
GVLFGLAYFSMVGNW,
TRVPYFVRAQGLIRA and
TTLLFNILGGWVAAQ.

9. HCV vaccine according to any one of claims 1 to 8 characterised in that it comprises the following epitopes:

GYKVLVLNPSVAAT,
DLMGYIPAV,
CINGVCWTV and
HMWNFISGIQYLAGLSTLPGNPA

10. HCV vaccine according to any one of claims 1 to 9, characterised in that it comprises the epitope KFPGGGQIVGGVYLLPRRGPRLGVRATRK.
11. HCV vaccine according to any one of claims 1 to 9, characterised in that it comprises the epitope DLMGYIPAV.
12. HCV vaccine according to any one of claims 1 to 9, characterised in that it comprises the epitope CINGVCWTV.
13. HCV vaccine according to any one of claims 1 to 9, characterised in that it comprises the epitope HMWNFISGIQYLGLSTLP-GNPA.
14. HCV vaccine according to any one of claims 1 to 9, characterised in that it comprises the epitope GYKVLVLNPSVAAT.
15. HCV vaccine according to any one of claims 1 to 9, characterised in that it comprises the epitope VVCCSMSYTWTGALITPC.
16. HCV vaccine according to any one of claims 1 to 15, characterised in that it further contains a peptide comprising a sequence $R_1\text{-XZ}_N\text{XZ}_N\text{-R}_2$, whereby N is a whole number between 3 and 7, preferably 5, X is a positively charged natural and/or non-natural amino acid residue, Z is an amino acid residue selected from the group consisting of L, V, I, F and/or W, and R_1 and R_2 are selected independantly one from the other from the group consisting of -H, -NH₂, -COCH₃, -COH, a peptide with up to 20 amino acid residues or a peptide reactive group or a peptide linker with or without a peptide; X- R_2 may be an amide, ester or thioester of the C-terminal amino acid residue of the peptide ("Peptide A").
17. HCV vaccine according to any one of claims 1 to 16, characterised in that it further contains an immunostimulatory oligodeoxynucleic acid molecule (ODN) having the structure according to the formula (I)



wherein

R1 is selected from hypoxanthine and uracile,

any X is O or S,

any NMP is a 2' deoxynucleoside monophosphate or monothiophosphate, selected from the group consisting of deoxyadenosine-, deoxyguanosine-, deoxyinosine-, deoxycytosine-, deoxyuridine-, deoxythymidine-, 2-methyl-deoxyinosine-, 5-methyl-deoxycytosine-, deoxypseudouridine-, deoxyribosepurine-, 2-amino-deoxyribosepurine-, 6-S-deoxyguanine-, 2-dimethyl-deoxyguanosine- or N-isopentenyl-deoxyadenosine-monophosphate or -monothiophosphate,

NUC is a 2' deoxynucleoside, selected from the group consisting of deoxyadenosine-, deoxyguanosine-, deoxyinosine-, deoxycytosine-, deoxyinosine-, deoxythymidine-, 2-methyl-deoxyuridine-, 5-methyl-deoxycytosine-, deoxypseudouridine-, deoxyribosepurine-, 2-amino-deoxyribosepurine-, 6-S-deoxyguanine-, 2-dimethyl-deoxyguanosine- or N-isopentenyl-deoxyadenosine,

a and b are integers from 0 to 100 with the proviso that a + b is between 4 and 150, and

B and E are common groups for 5' or 3' ends of nucleic acid molecules ("I-/U-ODN").

18. HCV vaccine according to any one of claims 1 to 17, characterised in that it further contains an $\text{Al}(\text{OH})_3$ adjuvant.

19. HCV vaccine according to any one of claims 1 to 18, characterised in that it further contains a polycationic peptide.

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20. HCV vaccine according to any one of claims 1 to 19, characterised in that said Peptide A is KLKL₅KLK.

21. HCV vaccine according to any one of claims 1 to 20, characterised in that said I-/U-ODN is oligo d(IC)₁₃.

22. HCV vaccine according to any one of claims 1 to 21, characterised in that it further contains an oligodeoxynucleotide containing a CpG-motif.

23. HCV vaccine according to any one of claims 1 to 23, characterised in that it is lyophilised in a form which is reconstitutable within 15 min. at 37°C.

24. HCV vaccine according to any one of claims 1 to 23, characterised in that it contains between 20 µg and 10 mg of each epitope.

25. HCV vaccine according to any one of claims 1 to 24, characterised in that it is lyophilised and contains traces of acetic acid.

26. HCV vaccine according to any one of claims 1 to 25 characterised in that it comprises at least two of the following epitopes:

KFPGGGQIVGGVYLLPRRGPRLGVRATRK, DLMGYIPAV, LEDRDRSELSPLLLSTTEW, DYPYRLWHYPCTVNFETIFKV, GYKVLVLNPSVAAT, CINGVCWTV, AAWYELT-PAETTVRLR, YLVAYQATVCARAQAPPPSWD, TAYSQQTRGLLG, HMWNFIS-GIQYLAGLSTLPGNPA, IGLGKVLVDILAGYGAGVAGALVAFK and SMSYTWGTGALITP.

27. HCV vaccine according to claim 25 characterised in that it comprises at least four, preferably at least five, especially at least six of these epitopes.

28. HCV vaccine according to claim 25 characterised in that it comprises at least eight, preferably all twelve of these epitopes.

29. HCV vaccine according to any one of claims 1 to 25 characterised in that it comprises at least two of the following epi-

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topes:

KFPGGGQIVGGVYLLPRRGPRLGVRATRK, DYPYRLWHYPCTVNFTIFKV
AAWYELTPAETTVRLR, TAYSQQTRGLLG, HMWNFISGIQYLAGLSTLPGNPA, IGLGK-
VLVDILAGYGAGVAGALVAFK and SMSYTWGALITP.

30. HCV vaccine according to claim 29 characterised in that it comprises at least four, preferably at least five, especially all twelve of these epitopes.

31. HCV vaccine according to any one of claims 1 to 30, characterised in that it comprises at least one A2 epitope, at least one DR1 epitope, at least one DR7 epitope or at least one of each of these epitopes.

32. Use of a vaccine according to any one of claims 1 to 31 for the preparation of a medicament for the prevention and treatment of an infection with HCV.

33. Method for the preparation of a vaccine according to any one of claims 1 to 31, characterised by the following steps:

- chemically synthesising the at least two epitopes as defined in claims 1 to 15 and 26 to 31,
- solubilising these epitopes by an aqueous solution containing at least one organic acid selected from the group consisting of formic acid, acetic acid, propionic acid, butyric acid and halogenated or hydroxylated forms thereof,
- mixing the solubilised epitopes and
- optionally lyophilising the mixed epitopes.